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ACTION OF SAPONIN ON THE BLOOD COR-
PUSCLES AND PUS CORPUSCLES

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A CONTRIBUTION TO OUR KNOWLEDGE OF THE ACTION OF SAPONIN ON THE BLOOD CORPUSCLES AND PUS CORPUSCLES.

By G. N. STEWART.

(From the Physiological Laboratory, Western Reserve University.)

I have shown in previous communications¹ that saponin produces a marked increase in the electrical conductivity of blood. The increase is quite as great in blood which has been fixed by formaldehyde as in fresh blood. It is, therefore, not dependent on the escape of the hæmoglobin from the corpuscles which saponin causes in unhardened blood, but not, of course, in blood treated with formaldehyde. I have demonstrated that no such effect is produced by saponin on blood-serum in the absence of the corpuscles. Its action on the conductivity of the blood may be explained in two ways: (1) It may increase the total conductance of the serum, (a) by causing the passage of electrolytes into it from the corpuscles without increasing its relative volume, or (b) by bringing about an increase in its volume, due to the passage of water out of the corpuscles and a consequent increase in the amount of dissociation of the electrolytes of the serum, or (c) by causing both water and electrolytes to pass out of the corpuscles.

(2) The saponin may increase the conductivity of the corpuscles without affecting that of the serum.

In the case of formaldehyde-hardened blood, where the action of saponin on the permeability of the corpuscles for electrolytes is most easily studied, since it is not complicated by the passage of the blood-pigment into the serum, it is not difficult to prove, by measuring the conductivity of the serum and sediment, respectively, after centrifugalization, that it is the conductivity of the corpuscles which is increased. If there is any increase in the conductivity of the serum,

¹ *Journ. of Physiology*, 1899, xxiv, p. 211, and 1901, xxvi, p. 470.

(a slight increase is perhaps indicated in some of the observations), it is small in comparison with the effect on the corpuscles. This is well seen in *Experiment I*,² in which the influence of saponin on the conductivity of unhardened blood is compared with that on blood fixed by formaldehyde. λ , as in the other experiments, is an abbreviation for $\lambda (5^\circ) \times 10^8$. The conductivities are expressed in reciprocal ohms reduced to 5° C. The measurements were made by Kohlrausch's telephone method, the tube containing the blood being immersed in running water, the temperature of which did not vary more than 2° or 3° from 5° C. The saponin solution used was made by dissolving 3 grammes crude quillaia saponin in salt solution and adding salt solution up to 100 cc. The salt solution had in some experiments a strength of about 0.7 per cent, in others of about 1 per cent. Its conductivity was always carefully measured, as was also that of the saponin solution, which did not differ much from the conductivity of the salt solution. As a control, observations were also made on blood to which, instead of saponin solution, a similar amount of the salt solution was added.

It will be seen that in *Exp. I*, in the observation of April 18, the serum from the mixture of A (the formaldehyde blood) and saponin has λ 83.66, while the serum from the corresponding mixture of A with the sodium chloride solution has λ 78.48. A portion even of the small difference shown here is accounted for by the greater conductivity of the saponin than of the sodium chloride solution. On the other hand, for the sediment of corpuscles from the mixture of A and saponin, after prolonged centrifugalization, λ is 46.51 against 18.68 for the sediment of the control experiment with A and sodium chloride, and 14.76 for the sediment of A. The great difference between the sediments is certainly not to be explained by the presence of a greater quantity of serum between the corpuscles in the sediment of the saponin mixture than in the others, for the sediment was exceedingly compact and well-separated from the serum. There can be no doubt, therefore, that saponin does render the corpuscles better conductors. From this it necessarily follows

² The protocols of experiments are at the end of this article (p. 272).

that the "envelope" of the corpuscle is rendered permeable to ions to which it refuses passage before the action of the saponin, or more rapidly permeable to those which penetrate it only slowly and with difficulty. But the experiment does not enable us to say whether, in addition, the saponin produces an increased dissociation inside the corpuscle.

I desire to point this out clearly because in a recent paper on the increase of conductivity which is known to occur in dying muscle,³ it is tacitly assumed that an increase of conductivity necessarily implies the formation of an increased number of ions in the muscle and is proportional to the increase in the number. Now while it is, of course, a perfectly familiar fact that when muscle dies a decomposition takes place which leads to the formation of substances capable of acting as conductors, we must not assume that this is the only way in which the conductivity is increased, until it is shown that no change in the resistance of the membranes through which the ions must pass is produced at death. The fact that the resistance of muscle is much greater in the transverse than in the longitudinal direction suggests very strongly that the resistance of the sarcolemma, and possibly also of whatever "envelopes" enclose the sarcostyles, is greater than that of the conducting liquids between the fibres or between the fibrils. And if those "membranes" are relatively impermeable to the electrolytes during the life of the tissue, as they should be if the proper osmotic relations are to be preserved, it is quite probable that their permeability, and therefore their resistance, becomes altered when the tissue dies. In this connection the fact mentioned in my previous paper, that perfectly fresh colored corpuscles are far less permeable to NH_4Cl than corpuscles which have stood for a few hours seems very suggestive.

I may mention here another observation which supports the conclusion that the envelopes of the corpuscles are altered by saponin. It was invariably found that when saponin was added at such an interval after the addition of formaldehyde that it no longer caused laking, the sediment of corpuscles settled far more slowly and less

³ T. Kodis, *American Journ. of Physiology*, 1901, v, p. 267.

perfectly, was always more bulky in proportion to the liquid above it, and formed a less compact and tenacious mass on the bottom of the test-tube than the sediment from formaldehyde blood which had not been treated with saponin or to which NaCl solution had been added in the same proportion as the saponin solution. This refers to the sediment obtained when the blood was simply allowed to stand in a cold room and not to that separated by prolonged centrifugalization. It was always much easier to shake up into complete suspension in the serum the sediment of the formaldehyde blood which had been acted on by saponin; and the greater the amount of saponin solution added, the looser and more easily shaken up was the sediment. The same effect was observed, though it was not so pronounced, in blood to which NH_4Cl solution was added in such amount as to cause partial laking, and which was then treated with formaldehyde. The sediment was distinctly looser than when NaCl was substituted for NH_4Cl in this combination. The suggestion is that NH_4Cl alters the envelopes of the corpuscles in such a way that they are less liable to adhere firmly to each other when acted on by formaldehyde, and that saponin undoes or lessens the formaldehyde action which causes them to adhere. The fact that NH_4Cl penetrates the corpuscles much more readily than NaCl also shows that its relation to the envelope is different. In this experiment it is incidentally shown that the preference of the corpuscles for NH_4Cl as compared with NaCl is still evident 15 days after the addition of formaldehyde to the blood.

Some leucocytes are of course always present in the sediments whose conductivity is compared in the experiment, which, however, yields no information as to the action of saponin upon these. But some observations on pus, to be presently described, show that saponin affects the conductivity of pus corpuscles in very much the same way as that of the red corpuscles. Since, as I have previously shown, the action of saponin on the red corpuscles is essentially the same whether they are perfectly fresh or have stood for days, it seems highly probable that the conductivity of the leucocytes of blood is also increased by saponin, and in the same way, that is, by an increase of their permeability to the ions of the serum. The "envelope" of

the leucocyte (or at least of the pus corpuscle), however, differs from that of the colored corpuscle, as we shall see, in exhibiting no preference for NH_4Cl as compared with NaCl .

In *Experiment II* the effect of varying the quantity of saponin added to formaldehyde blood on its conductivity was investigated. Under λ the conductivities of the mixtures of formaldehyde blood and saponin are given, and in the column headed " λ of control" the conductivities of mixtures of the formaldehyde blood with similar quantities of the NaCl solution used in making the saponin solution. In the third column the differences between λ and λ of control are given, that is to say, the approximate amounts by which the conductivity of the blood has been increased by the action of the saponin, after deducting the increase due to the NaCl in the saponin solution. I shall speak of the numbers in this column as the true saponin effect.

The experiment shows that the addition of 1 cc. of the saponin solution (the largest quantity used) to 5 cc. of the formaldehyde blood (A) causes practically the same true saponin effect as the addition of 0.4 cc. of the saponin solution to 5 cc. of A. In both cases, too, the full increase of conductivity is already developed at the time of the first measurement (a few minutes after mixture), and there is no further increase when the mixture is allowed to stand for many hours. When 0.2 cc. of the saponin solution is added to 5 cc. of A the true saponin effect ultimately becomes just as great as when 0.4 cc. or 1 cc. is added, but it has not reached its maximum 12 minutes after mixture. Eight hours after mixture the effect is complete, and there is no further increase after many hours. With 0.1 cc. of saponin solution to 5 cc. of A the true saponin effect is only 14.79, 20 minutes after mixture, as compared with 22.14, 19 minutes after the addition of 0.4 cc. of saponin solution to 5 cc. of A. But in the observation with 0.1 cc. of saponin solution the effect has not reached its maximum even 2 hours and 20 minutes after mixture, when it is 16.23. In 19 hours more it has further increased to 18.05. With 0.05 cc. of the saponin solution to 5 cc. of A the increase in the true saponin effect, as time goes on, is still more striking, the effect being only 5.39, 27 minutes after mixture, but 8.56, $4\frac{1}{2}$ hours after mixture, and 9.31,

16 hours later. The maximum effect remains much less for this quantity of saponin than for 0.1 cc. or anything above it. With 0.02 cc. of the saponin solution (the smallest quantity employed) to 5 cc. of A the maximum effect is still less, but the relative increase between the first and last measurement is the greatest of all (from 0.74 to 3.33).

The action of saponin in laking the colored corpuscles has usually been ascribed to a solution or "corrosion" of some constituent of the stroma (or envelope). The fact that it greatly increases the conductivity of the formaldehyde-hardened corpuscles is an additional argument in favor of this view. It further shows that the substance on which saponin acts is not fixed by formaldehyde, within the longest period investigated (a fortnight), or at least is not altered in such a way as to become incapable of being attacked by saponin. This makes it highly probable that the substance is not a proteid. Ransom⁴ has recently brought forward evidence that the substance on which saponin acts when it lakes the corpuscles is cholesterin. My results are compatible with this idea. But it seems certain that if the saponin produces its effect on the conductivity of formaldehyde blood by attacking the cholesterin in the corpuscles, the result is due not to any merely chemical reaction between the saponin and the cholesterin, but to a change of structure in the corpuscles which renders them more permeable to ions and perhaps in addition to an increased dissociation of electrolytes in the corpuscles. I have not hitherto found anything in the appearance of the corpuscles under the microscope or their behavior to stains which throws any light on the point of attack of the saponin.

In *Experiment III* I endeavored to determine whether the addition of saponin to yolk of egg, which is known to be rich in cholesterin (and lecithin) compounds would affect the conductivity at all in the same way as it affects the conductivity of blood. I found that it had no more influence on the yolk than on the white, and no greater effect on either than was due to the electrolytes contained in the saponin solution. On the other hand it was shown that there was a marked difference in the effect of dilution with water on the conduc-

⁴ *Deutsche med. Wochenschr.*, 1901, p. 194.

tivity of the white and the yolk respectively, the dilution causing a much smaller proportional diminution in the conductivity of the yolk than in that of the white. Thus in *Experiment IV* the conductivity of the yolk was 19.83 and that of the white 56.46. Dilution with 1 volume of water (Fig. 1) reduced the conductivity of the former to 17.76 and that of the latter to 33.98; while the addition of 3 volumes of water made the conductivity of the yolk 11.52 and that of the white 19.26. The conductivity of the undiluted yolk is always much less than that of the undiluted white, the ratio being usually about 1:3.

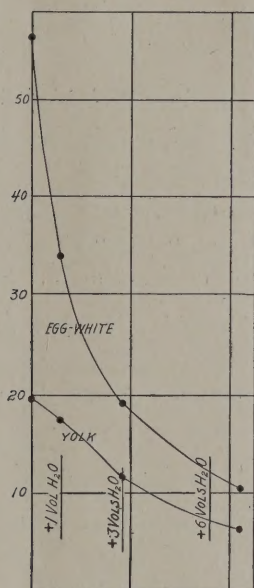


FIG. 1.

Curves showing the effect of dilution with water on the conductivity of yolk and of white of egg, plotted from Exp. IV. Conductivities laid off along vertical axis, and quantities of water added along horizontal axis.

The fact that saponin increases the conductivity of blood-corpuscles which have been fixed by formaldehyde suggests that its primary action on unhardened blood is the same, viz., an increase in the permeability of the envelope to the electrolytes of the serum or of the corpuscles themselves. The osmotic equilibrium would be thus

upset; some of the water of the serum would pass into the corpuscles; and when the increase of permeability reached a certain limit enough water might enter to cause of itself the discharge of the hæmoglobin. Saponin-laking would thus be reduced to a special form of water-laking, as Nolf⁵ and Hédon⁶ have suggested. My experiments, in so far as they demonstrate an actual increase of permeability for the electrolytes, are distinctly in favor of this hypothesis. Support is also lent to this view by the fact that an amount of saponin insufficient to produce laking may cause the blood to darken, the corpuscles swelling up without losing hæmoglobin. This change is not accompanied by the marked increase of conductivity which signalizes the actual onset of the laking process, although the conductivity may be somewhat increased.

Another fact, which, if it does not so directly support this theory of the saponin action, is at least consistent with it, is that the laking action of saponin and also its effect on the conductivity are much more easily produced at a temperature of 40° to 45° C. than at the ordinary temperature. A dose of saponin which may be ineffective at room temperature may cause complete laking and the characteristic increase of conductivity when the blood is heated to the temperature mentioned, and a dose which may be just capable of laking slowly at ordinary temperature will bring about rapid laking at 40° to 45°. Occasionally I have noticed, as in *Exp. V*, that a dose to which blood is refractory at 40° to 45°, becomes rapidly effective at 50° C. The phenomenon is different from heat-laking, for this cannot be obtained even by prolonged heating at such temperatures. The action seems to be simply the ordinary saponin action intensified by the increase of temperature, which may diminish the power of resistance of the envelope to the attack of the saponin and the penetration of the electrolytes.

A diminution in the resistance of the corpuscles to the action of saponin may be actually observed when blood is allowed to stand for a while before addition of the saponin. A given dose of saponin may

⁵ *Ann. de l'Institut Pasteur*, 1900, p. 297.

⁶ *Archives internat. de pharmacodynamie et de thérapie*, 1901, viii, p. 403.

then produce a greater increase of conductivity and more complete laking than the same dose produced in the fresh blood; and a dose which is insufficient to bring about complete laking, with the corresponding increase of conductivity, in fresh blood, may do so in stale blood.

With the view of throwing further light on the changes which occur in the serum and the corpuscles when unhardened blood is laked by saponin—a subject, as already remarked, less easy to study than the corresponding changes in formaldehyde blood—I have compared the conductivity and freezing-point of ordinary defibrinated blood and of formaldehyde blood before and after the addition of saponin. The same quantities were determined for the sera of the defibrinated blood, the formaldehyde blood and the mixture of the latter with saponin (*Experiment V*). It was found impracticable to separate the ghosts from the saponin-laked defibrinated blood by the centrifuge, and therefore in this case the freezing-point was measured directly on the laked blood, and the conductivity of the corpuscle-free liquid was not determined. But in *Experiment VI* the ghosts were completely separated from saponin-laked blood by filtration through a pot of porous clay, and the conductivity of the liquid measured.

In *Exp. V* from the percentage of serum in the defibrinated blood (estimated by the electrical method previously described by me⁷) and the actual Δ of the serum and saponin solution I have calculated the value of Δ for the extra-corpuscular liquid of the mixture of blood and saponin, on the assumption that no exchange of water or dissolved solids between the corpuscles and this liquid is caused by the saponin, or that, if there is any exchange, water accompanies the solids in such proportion as to leave the Δ of the extra-corpuscular liquid the same as if no exchange took place.

From the percentage of extra-corpuscular liquid⁸ in the formaldehyde

⁷ *Journ. of Physiology*, 1899, xxiv, p. 356.

⁸ I have supposed for the purpose of calculating this percentage that the formaldehyde solution when added to blood simply mixes with the serum, without any alteration in the volume of the serum-formaldehyde mixture taking place through exchange with the corpuscles. Since the corpuscles preserve their normal size and shape this assumption is approximately correct. But although there is no notable movement of

blood A and the actual λ and Δ of this liquid and of the saponin solution I have, on the same assumption, calculated the value of λ and Δ for the extra-corpuscular liquid of the mixture of A and saponin. In calculating λ and Δ I have made no allowance for possible changes in the dissociation coefficient when mixture takes place. Comparison of the actual and calculated values of λ and Δ , of course, affords information as to the correctness of the assumption on which the calculation is based, and where it is erroneous indicates how it must be modified.

It will be seen that for the mixture of defibrinated blood and saponin the actual is distinctly greater than the calculated Δ . This is doubtless due in part to the escape of hæmoglobin into the serum. But the molecular weight of hæmoglobin is so great that even the solution of the whole of the hæmoglobin would not suffice to account for the difference. In *Experiment VI* the liquid obtained by filtering off the ghosts from saponin-laked blood had a conductivity about equal to that of the serum of the original defibrinated blood, although decidedly less than that of serum obtained from blood to which as much of the NaCl solution used in making the saponin solution had been added as was added of the saponin solution to the saponin blood. The deficiency in λ of the serum of the saponin-laked blood is easily explained by the depressing influence on the conductivity exerted by the dissolved hæmoglobin. Indeed the quantity of hæmoglobin in solution would cause a considerably greater deficiency than actually exists. So that it seems necessary to suppose that water has entered the corpuscles from the serum in greater proportion than electrolytes, or that if water has come out of the corpuscles it has been accompanied by electrolytes in such amount that, apart from the depressing influence of the hæmoglobin, the conductivity of the serum would have been increased. The latter alternative would also explain the excess of the actual over the calculated Δ in *Exp. V*, and it is this that we must accept. For although at the beginning of the saponin action water seems to enter the corpuscles and to cause them to swell, the process must be reversed with the discharge of the hæmoglobin, since in saponin-laked blood the ghosts constitute only a relatively small proportion of the total volume. We must, therefore, suppose that as the hæmoglobin escapes from the corpuscles, they also lose water and electrolytes.

water into or out of the corpuscles, much of the formaldehyde certainly enters them. This is well illustrated by the fact that for the serum from A the calculated is much greater than the actual Δ , while the actual λ is almost the same as the calculated. This shows that the difference between the actual and calculated Δ is due to the passage of non-electrolytes, and not of electrolytes into the corpuscles.

In the case of the extra-corpuscular liquid of the mixture of formaldehyde blood and saponin the difference between the actual and calculated Δ is too great to be explained by the passage even of all the saponin into the corpuscles. It is most easily accounted for by the supposition that saponin increases the permeability of the corpuscles for certain of the dissolved substances in the extra-corpuscular liquid. If any salts pass into the corpuscles, water must enter along with them, or other salts must escape from the corpuscles, since the actual λ of the corpuscle-free liquid (referred to as "top" in the tables) is practically the same as the calculated λ . The difference between the actual and calculated Δ must accordingly be due to the passage not of electrolytes but of non-electrolytes (formaldehyde) into the corpuscles. As has been shown in *Exp. I*, the marked increase of λ of the entire formaldehyde blood under the influence of saponin is due to an increase in the permeability of the corpuscles for ions. I have already given reasons for supposing that when ordinary (unhardened) blood is acted on by saponin the corpuscles become more permeable to the salts of the serum, or certain of them, and presumably to their ions. Whether this increase of permeability persists when the corpuscles have been reduced to ghosts by the escape of the hæmoglobin I am unable to say. The ghosts certainly remain worse conductors than the serum, as is shown by the fact that as the serum is separated from saponin-laked blood the conductivity of the residue sinks (see e. g. *Exp. VI*).

Another point of interest illustrated in *Exp. V*, and still better in *Exp. I*, is that the conductivity of a specimen of defibrinated blood treated first with formaldehyde and then after a while with saponin is not in general the same as the conductivity of another specimen of the same blood to which saponin is first added and then formaldehyde in the same amount as in the first observation. The difference, of course, depends on the presence of the corpuscles, and would not be seen if the electrolytes of the blood were all in simple solution. The combination blood + saponin + formaldehyde has in general a smaller conductivity than the combination blood + formaldehyde + saponin, especially when in the second combination blood which has been acted on so long by the formaldehyde that no laking takes place on the addition of saponin is employed. This supports the conclusion already drawn, that one of the causes of the smaller conductivity of the first combination is the depressing effect of the hæmoglobin on the conductivity of the laked blood. In addition, it is probable that a great part of the formaldehyde, which, as a non-conductor, depresses the conduc-

tivity of the extra-corpuscular liquid, is in the second combination fixed in the corpuscles.

THE BEHAVIOR OF PUS CELLS TO NH_4Cl , NaCl , SAPONIN AND WATER.

In order to see whether other cells, and especially dead cells, would exhibit any of the peculiarities studied in the colored corpuscles, I made some experiments with pus.

In *Experiment VII* it is shown that pus corpuscles, like colored blood-corpuscles, have a smaller conductivity than the serum of pus. The serum can be separated from the corpuscles by filtering the pus through porous clay, but more easily, and as completely, or nearly so, by filtering it through paper, putting the first portion of the filtrate back on the filter. I have had no success in obtaining the serum free from corpuscles by the centrifuge. On the average the conductivity of the pus serum is very much the same as that of blood serum, but sometimes it is considerably greater, especially, of course, if putrefaction is at all pronounced. I have previously shown⁹ that in putrefying liquids the conductivity increases and the freezing-point diminishes progressively up to a certain limit, as time goes on, owing to the conversion of proteids or other complex substances into simpler compounds, some of which are electrolytes. I have never obtained such small values for the conductivity of sediments rich in pus cells as for blood sediments, and, therefore, it would seem that pus corpuscles are more permeable to the ions of the serum than blood-corpuscles are. But, as is seen in *Exp. VII*, the conductivity of the sediment is much less than that of the serum.

When diluted with water (*Exp. VII* and Fig. 2) pus has a higher conductivity than a simple solution of electrolytes or blood-serum or egg-white diluted to the same extent, but a lower conductivity than correspondingly diluted yolk of egg (*Exp. IV* and Fig. 1) or defibrinated blood. This indicates that electrolytes may pass out of the corpuscles when pus is diluted with water. But in the absence of measurements of the conductivity and freezing point of the corpuscle-free liquid from the diluted pus one cannot make any positive statement as to this. For there are three other factors which would cause

⁹ *Journal of Experimental Medicine*, 1899, iv, p. 235.

a relative increase of conductivity: increased dissociation of electrolytes in the serum, diminution of the depressing effect of the non-electrolytes, and increase in the average breadth of the conducting paths between the corpuscles.

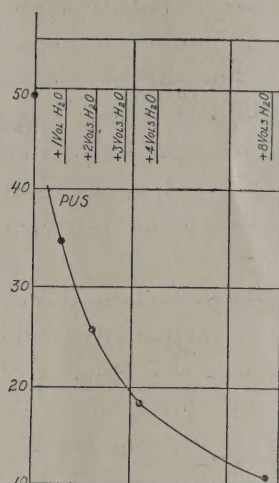


FIG. 2.

Curve showing the effect of dilution with water on the conductivity of pus, plotted from Exp. VII. Conductivities along vertical, quantities of water added along horizontal axis.

Heating pus to 69° C. (*Exp. VII*) and subsequent dilution failed to reveal any analogy between the behavior of pus corpuscles and that of blood corpuscles, the ratio between the conductivity of the pus which had been heated and that of the heated pus after dilution with its own volume of water being practically the same as the corresponding ratio for a specimen of unheated pus and for a sediment of pus. In *Exp. IX* it is shown that when pus is heated to 80° C. for 10 minutes its conductivity is increased. The increase is still greater when the pus is heated to 93° for 15 minutes, although the conductivity of the serum is diminished.

On the other hand, saponin, as is shown in *Experiment VIII*, increases the conductivity of pus just as it does the conductivity of blood. This is the case also when saponin is added to pus which has been treated with formaldehyde. The action is on the corpuscles, since the

addition of saponin to pus serum causes only the change of conductivity due to the electrolytes in the saponin solution (*Exp. IX*). When saponin is added to pus which has been heated to 93° C. (*Exp. IX*) the characteristic increase of conductivity is still produced, although the proteids of the corpuscles must have been coagulated. With the microscope the corpuscles, or at least great numbers of them, were seen to be still intact, and stained normally. These facts are in accordance with what has been already mentioned as to the action of saponin on formaldehyde blood, and suggest that in the case of pus also the constituent of the corpuscles on which the saponin acts is not of proteid nature.

At the same time it ought to be pointed out that the entire increase produced by saponin in the conductivity of the heated pus is not due to an action on the corpuscles; a portion of the increase is caused by some action of the saponin on the serum. This is proved in *Experiment IX*, where the behavior to saponin of the serum of unheated pus is compared with that of the serum of the same pus after heating to 93° . In the case of the serum of the unheated pus the saponin, as already stated, produces only the small increase of conductivity corresponding to the electrolytes in the saponin solution. The increase in the conductivity of the serum of the heated pus is too great to be entirely accounted for in this way. I have no results as yet which would enable us to decide whether the action of the saponin on the serum of the heated pus causes the granules present in it (some of which may have escaped from corpuscles either partially or wholly broken down by the heating) to become better conductors, or whether the increase in conductivity is due to the action of the saponin on dissolved substances. It cannot be due to any action on intact corpuscles in the serum, for it was shown by the microscope that none such were present.

There is no evidence (*Exp. VIII*) that pus corpuscles take up NH_4Cl in preference to NaCl either in the case of normal pus or of pus hardened by formaldehyde.

SUMMARY.

1. The increase of conductivity produced by saponin in formaldehyde-hardened blood is due to an increase in the conductivity of the

corpuscles (increased permeability of the corpuscles to ions) and not, mainly at any rate, to the liberation of electrolytes from the corpuscles and a consequent increase in the conductivity of the serum. The increase in the permeability of the corpuscles is probably caused by a "corrosive," dissolving, or emulsifying action of the saponin on some non-proteid constituent of the envelope or stroma.

2. In the first stage of the action of saponin on blood (not fixed by formaldehyde) there seems also to be an increase in the permeability of the corpuscles for ions, even before any hæmoglobin has been liberated. The liberation of the hæmoglobin may be secondary to this, owing to the entrance of water consequent on the disturbance of osmotic equilibrium.

3. Heating the blood to 40° to 45° C. intensifies the laking action of saponin, so that a dose insufficient to cause laking at ordinary temperature may do so when the blood is heated to the temperature mentioned.

4. Pus corpuscles, like red blood corpuscles, are worse conductors than the serum in which they are suspended. Unlike blood corpuscles, they show no preference for NH_4Cl as compared with NaCl . On the other hand, the conductivity of pus is increased by the action of saponin, just as is the case with blood, and apparently very much in the same way, that is to say, by an action on the corpuscles and not on the serum. The fixing of the pus corpuscles by formaldehyde does not hinder this action of saponin.

PROTOCOLS OF EXPERIMENTS.

EXP. I.

April 17. At 10.20 A. M. obtained defibrinated blood from a bitch. At 3.50 P. M. added to 150 cc. of the defibrinated blood 75 cc. of a 4 per cent solution of formaldehyde in NaCl solution. The mixture is called A. For the formaldehyde solution $\lambda=64.18$; for the saponin solution $\lambda=83.94$; for the NaCl used in making the saponin and formaldehyde solutions $\lambda=78.97$. Percentage of serum in the defibrinated blood calculated by the electrical method, 51.5.

Time.	Defibrinated blood.	λ	Time.	Formaldehyde blood A.	λ
April 17, P. M.			April 17, 8.37 P.M.	A	37.01
8.30	Defibrinated blood	30.18	7.38	30 cc. A + 1.6 cc. saponin sol.	
7.17	20 cc. defib. blood + 1.6 cc. saponin.		7.45	Considerably darkened.	
7.23		34.63	8.23	Well laked	56.33
7.45	Not laked, only somewhat darkened; heated in bath at 44° to 45° along with A + saponin. A + saponin lakes far sooner and more completely than defib. blood + saponin.			(After standing 18 hrs. is thick and viscid)	53.26
			April 18, P. M.		
8.10	Defib. blood + saponin (well laked) ..	37.95	3.38	30 cc. A + 1.6 cc. saponin sol.	
8.14	To the mixture of defib. blood and saponin added 10 cc. formald. sol.		6.20	Not laked	58.41
8.45	This mixture	43.71		(After standing 13 hrs. is still thin and not laked)	59.79
	(After standing 18 hours)	44.55		Top (after centrif. for 50 min.) ..	83.66
April 18, P. M.				Bottom	46.51
3.05	Defibrinated blood	28.87		A + as much of the NaCl sol. used in making the saponin solution as was added of the saponin sol.	
	Serum from clot	84.22		(After standing 22 hrs.)	41.90
2.55	20 cc. blood + 1.6 cc. saponin.			Top (after centrif. for 20 min) ..	78.48
3.17	Only somewhat darkened; not at all laked.			Bottom	18.68
4.25	Not laked, although dark.			Top of A (after centrif. for 50 m.)	79.72
6.15	Now laked	43.11		Bottom	14.76
6.20	Added to this mixture of defib. blood and saponin 10 cc. formald. sol.		April 19, 8.01 P.M.	15 cc. A + 1.2 cc. saponin sol.	
6.37	This mixture	47.56	8.05		58.95
	(After standing 13 hrs., perfectly laked and still thin)	46.86		(After standing 18 hrs.)	61.38
	Bottom* (after centrif. 20 min.) ..	47.47		Top (after centrif.)	80.22
3.17	20 cc. blood + 1.6 cc. of the NaCl sol. used in making the saponin sol.		8.12	10 cc. A + 20 cc. water.	
6.05		32.09	8.17		23.02
	(After standing 13½ hrs.)	29.55		(After standing 17 hrs.)	23.78
				Top	30.11
				Bottom	13.60
				Bottom (after centrif.)	7.52
				A	35.96
			8.25		
			May 2.		
			A. M.		
			10.49	A	38.53
			11.00	5 cc. A + 0.4 cc. saponin sol.†	
			11.19		66.53
			P. M.		
			7.53		66.19
				(19 hours later)	66.36
			A. M.		
			11.00	5 cc. A + 0.4 cc. of the NaCl sol.‡ used in making the saponin sol.	
					
			11.11		44.09
			11.20	5 cc. A + 5 cc. NH ₄ Cl§	
				(After standing 24 hours	74.34
				Top	103.47
			11.20	5 cc. A + 5 cc. NaCl †	
				(After standing 24 hours)	80.22
				Top	111.18

* There is very little separation. The ghosts cannot be separated by the centrifuge but can be completely separated by filtration through paper. After a time both the filtrate and the sediment on the filter set into a very firm jelly.

† For this saponin solution $\lambda = 139.30$.

‡ For the NaCl solution $\lambda = 140.07$.

§ For the NH₄Cl solution $\lambda = 143.10$.

EXP. II.

To show effect of different quantities of saponin on the conductivity of formaldehyde blood. Formaldehyde blood A (from Exp. V) was used. On April 17, 75 cc. of a 4 per cent solution of formaldehyde in NaCl solution was added to 150 cc. of bitch's defibrinated blood. For the saponin solution $\lambda=139.30$. For the NaCl solution used in making the saponin solution $\lambda=140.07$.

Time.		λ	λ of control.	Difference bet. λ and λ of control.
May 2,				
10.49 A. M.	A	38.53		
11.00	5 cc. A + 0.4 cc. saponin.			
11.19		66.53	44.09	22.44
7.53 P. M.		66.19		22.10
	(19 hours later).....	66.36		22.27
11.50 A. M.	5 cc. A + 0.2 cc. saponin.			
12.02		62.44	41.35	21.09
8.01 P. M.		63.85		22.50
	(19 hours later).....	63.85		22.50
3.13 P. M.	5 cc. A + 0.1 cc. saponin.			
3.33		54.52	39.73	14.79
8.08		55.96		16.23
	(19 hours later).....	57.78		18.05
3.50	5 cc. A + 0.05 cc. saponin.			
4.17		43.86	38.47	5.39
8.15		47.03		8.56
	(16 hours later).....	48.19	38.88	9.31
3.50	5 cc. A + 1 cc. saponin.			
4.23		73.91	51.11	22.80
8.23		72.84		21.73
	(16 hours later).....	72.84		21.73
4.35	5 cc. A + 0.02 cc. saponin.			
4.50		39.80	39.06	0.74
8.30		40.95		1.89
	(15½ hours later).....	42.39		3.33

EXP. III.

White and yolk of two fresh hen's eggs, beaten up separately and strained through muslin. For the saponin solution $\lambda=140.83$; for the NaCl solution used in making it, $\lambda=141.62$.

	λ		λ
White	59.09	Yolk	21.45
5 cc. white + 0.8 cc. saponin solution	69.26	5 cc. yolk + 0.8 cc. saponin solution	35.06
5 cc. white + 0.81 cc.* of the NaCl sol. used in making the saponin sol.	69.07	5 cc. yolk + 0.81 cc.* of the NaCl sol. used in making the saponin sol.	35.36

* .01 cc. too much was added by mistake.

EXP. IV.

White and yolk of hen's egg beaten up separately.

	λ	Ratio of λ of undiluted to λ of diluted.		λ	Ratio of λ of undiluted to λ of diluted.
White.....	56.46		Yolk.....	19.83	
+1 vol. water..	33.98	1.66	+1 vol. water..	17.76	1.11
+3 " " ..	19.26	2.93	+3 " " ..	11.52	1.72
+7 " " ..	10.41	5.42	+7 " " ..	6.78	2.92

To dog's defibrinated blood, two hours after it was drawn, one-half of its volume of a 4 per cent solution of formaldehyde in a 1 per cent NaCl solution was added. Call the mixture A. The measurements in the table were begun 18 hours later. The animal had been used for intravenous injection of glycerine extract of suprarenal capsule one hour before the blood was obtained. For the formaldehyde solution $\lambda = 111.18$; $\Delta = 3.755$. For the NaCl solution $\lambda = 139.30$; $\Delta = 0.732$. For the saponin solution $\lambda = 134.84$; $\Delta = 0.851$. The proportion of serum in the defibrinated blood as calculated by the electrical method is 60.6 per cent. The proportion of serum (extracorporeal liquid) in A, calculated on the assumption that the addition of the formaldehyde caused neither loss nor gain of water by the corpuscles, is 73.7 per cent.

FORMALDEHYDE BLOOD (A).

DEFIBRINATED BLOOD.

Time.	P. M.	λ actual.	λ calculated.	Δ actual.	Δ calculated.	Time.	λ actual.	λ calculated.	Δ actual.	Δ calculated.
1.18		Serum from defibrinated blood...	85.93		0.761	P. M.		Serum from A (practically free from blood pigment).....	96.75	2.11
4.22		Defibrinated blood.....	38.47			1.32		A.....	61.53	
2.59		To 20 cc. of defib. blood added 1.6 cc. saponin solution.				2.21		To 25 cc. of A added 1.33 cc. of the saponin sol.		
6.10		No laking. Added 0.4 cc. more of the saponin solution.				2.27		No laking. Added 0.4 cc. of the saponin sol.		
6.35		Not laked. Heated to 44° for 15 min. Darkens somewhat, but is not laked.				6.10		Not laked. Heated to 44° for 15 min. No change.		
6.50		Added 0.4 cc. more of the saponin sol. Left at 45° for 16 min.; is darkened but not laked.				6.35		Added 0.4 cc. more of the saponin solution, and left in bath at 45° for 16 min.		
7.25		Added 0.4 cc. more of the saponin solution.				6.50		Added 0.4 cc. more of the saponin solution.	97.3	1.686
7.35		Not laked. Heated to 50° for 10 min.; lakes almost at once.				7.25		Not laked. Heated to 50° for 10 min.; although darker than A, it is not laked.		
7.54		Seems perfectly laked. (After standing 23 hrs. more)...	56.58 66.36		0.799	7.35		Not laked. (After standing 15½ hrs. more).	80.22 81.25	
8.35		Serum from mixture of blood and saponin.....		95.10		8.05		Top (after centrifugation).	102.22	1.585
9.03		To 11.4 cc. of the mixture of blood and saponin added 5 cc. of the formaldehyde solution. (Quite thick, but can still be poured out).....	75.00		1.97				101.3	1.427
		In 4 min. more it set completely into a jelly, so that Δ could not be determined.								
		Top.....		101.5						

EXP. VI.

Dog's defibrinated blood. For the saponin solution $\lambda=140.83$; for the NaCl solution used in making it $\lambda=141.62$.

	λ
The defibrinated blood	33.75
Serum from clot (free from haemoglobin)	88.33
To 50 cc. of defibrinated blood added 4 cc. of saponin solution (dark and laked in 5 minutes)	68.51
Filtered the saponin blood several times through paper, and centrifugalized the filtrate.	
Filtrate (contains many ghosts and leucocytes)	71.01
Filtered this filtrate through a pot of porous clay.* (Now free from corpuscles)	88.64
Residue containing the ghosts and leucocytes but also much serum ...	67.78
To 25 cc. of defibrinated blood added 2 cc. of the NaCl solution used in making the saponin solution. This mixture	38.47
Serum from this mixture (free from corpuscles)	98.26

* It filters much more slowly than water-laked blood, obtained by adding 2 volumes water to the same defibrinated blood, and filtered under the same pressure.

EXP. VII.

	λ	Ratio of λ of pus to λ of diluted pus.
Pus from tubercular hip joint	49.51	
+1 vol. water	34.12	1.45
+2 "	25.66	1.92
+3½ "	18.56	2.66
+8 "	10.39	4.75
Clear serum from pus (filtered through clay)	86.52	
Fresh pus obtained on another occasion from the same hip joint	57.61	
Clear serum (filtered through paper)	77.52	
Sediment	56.58	
Sediment +1 vol. water	39.45	1.43
" +3 vols. water	22.04	2.56
Another specimen of pus	49.56	
Clear serum from it (filtered through paper)	91.30	
Sediment	38.55	
Another specimen of pus	49.94	
Heated to 69° C. (became thicker than original pus)	50.49	
Heated pus +1 vol. water	34.87	1.44

EXP. VIII.

Pus from a case of empyema obtained May 7. On May 8, at 8 p. m., added to 50 cc. of the pus 20 cc. of a 4 per cent solution of formaldehyde in NaCl solution. Call the mixture A. For the formaldehyde solution $\lambda=123.66$. For the saponin sol. $\lambda=140.83$; for the NaCl solution $\lambda=141.62$; for the NH_4Cl solution $\lambda=143.90$.

Time.	Pus.	λ	Time.	Formaldehyde pus (A).	λ
May 8, P. M.			May 9, P. M.		
8.09	Pus.....	46.51	4.26	A.....	66.19
	Serum separated by filtration....	109.27		Serum from A.....	93.54
7.36	10 cc. pus+10 cc. NH_4Cl .		4.40	10 cc. of A+10 cc. NH_4Cl .	
8.19		89.58	7.19		101.81
	(After standing 12 hours more)..	89.26	4.40	10 cc. of A+10 cc. NaCl.	
	Top	117.91	7.25		101.00
7.37	10 cc. pus+10 cc. NaCl.		4.49	5 cc. of A+0.4 cc. saponin.	
8.25		89.26	7.32		78.48
	(After standing 20 hours more)..	88.64	4.49	5 cc. of A+0.4 cc. of the NaCl solution used in making the saponin sol.	
	Top	116.28	7.40		70.03
7.41	10 cc. pus+0.8 cc. saponin.		4.52	5 cc. of A+10 cc. of water.	
8.31		59.65	7.48		32.67
	(After standing 11½ hrs. more)..	61.98		(After standing 16 hrs.) Top ..	38.82
7.42	10 cc. pus+0.8 cc. of the NaCl sol. used in making the saponin solution.		May 14, A. M.		
8.37		52.27	11.47	5 cc. of A+0.4 cc. of saponin.	
7.45	10 cc. pus+20 cc. water.		P. M.		
8.49		29.34	12.07		80.47
9.10	10 cc. pus+1.6 cc. saponin.		A. M.		
	(After standing 10½ hrs.)	74.34	11.47	5 cc. of A+0.4 cc. of the NaCl solution used in making the saponin sol.	
9.10	10 cc. pus+1.6 cc. of the NaCl sol. used in making the saponin solution.		12.00		71.41
	(After standing 10½ hrs.)	56.08			

EXP. IX.

Same pus as that used in Exp. VIII obtained on May 7. The solutions of NH_4Cl , and NaCl, saponin are the same as those used in Exp. VIII.

Time.		λ	λ of control *
May 14, 3.00 P. M.	Pus heated to 80° C. for 10 minutes	56.71	
3.10	5 cc. of the heated pus+0.4 cc. saponin.		
3.28		63.69	61.23
3.48	The original pus (not heated)	52.81	
4.00	Pus heated to 93° C. for 10 minutes	61.23	
4.20	5 cc. of this heated pus+0.4 cc. saponin.		
4.41		68.14	64.67
May 15, 2.30 P. M.	Clear serum from original pus (filtered through paper, entirely free from pus corpuscles)	109.27	
2.42	5 cc. of this serum+0.4 cc. saponin.		
2.59		111.67	111.67
7.21	Serum from pus which was heated to 93° (sepa- rated by centrifuge. Very few pus corpuscles in it, but a good many granules)	95.66	
7.16	5 cc. of this serum+0.4 cc. saponin.		
7.45		105.63	101.81
May 16, 10.53 A. M.	Serum from pus which was heated to 93° (sepa- rated by filtration through paper. Entirely free from pus corpuscles. Not many granules can be seen till methylene blue is added, when a good many appear, many of which are cocci)	93.54	
11.10	5 cc. of this serum+0.4 cc. saponin.		
11.26		106.51	98.26

* In this column are given the conductivities of pus or serum of pus to which instead of saponin solution the corresponding amount of the NaCl solution used in making the saponin solution was added as a control.

